Supporting Information

Emission Wavelength Switchable Carbon Dots Combined with Biomimetic Inorganic Nanozymes for Two-Photon Fluorescence Immunoassay

Yuanjin Zhan,[a] Shuangting Yang,[a,b] Fang Luo,[c] Longhua Guo,[b*] Yanbo Zeng,[b*] Bin Qiu,[a] and Zhenyu Lin[a*]

[a] Institute of Nanomedicine and Nanobiosensing; MOE Key Laboratory for Analytical Science of Food Safety and Biology; Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety; College of Chemistry, Fuzhou University, Fuzhou, 350116, China.

[b] College of Biological, Chemical Sciences and Engineering, Jiaxing University, Jiaxing, Zhejiang 314001, China.

[c] College of Biological Science and Engineering, Fuzhou University, Fuzhou, Fujian 350116, China.

Corresponding Author

[*]E-mail: guolh@zjxu.edu.cn (LH Guo); ybzeng@mail.zjxu.edu.cn (YB Zeng); zylin@fzu.edu.cn (ZY Lin).

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EXPERIMENTAL SECTION

Reagents and materials. *o*-phenylenediamine (*o*PD), triethanolamine (TEOA), sodium salicylate, (3-aminopropyl)triethoxysilane (APTES), and tetraethyl orthosilicate (TEOS) were purchased from Aladdin Chemical Industries, Ltd (Shanghai, China). $FeSO_4$, $FeCl_3$, H_2O_2, glucose, hexadecyl trimethyl ammonium bromide (CTAB), Na_2CO_3, NaCl, NaN_3, ethanol, hydrochloric acid (HCl), chloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), tris(hydroxymethyl)aminomethane (Tris), glutaraldehyde solution (50%), and 3,3',5,5'-tetramethylbenzidine (TMB) were obtained from Sinopharm Group Co., Ltd (Shanghai, China). Bovine serum albumin (BSA) was achieved from Sangon Biotech Group Co., Ltd. (Shanghai, China). TWEEN-20 was acquired from ABCONE Biotech Group Co., Ltd. (Shanghai, China). Human carcinoembryonic antigen (CEA, cat#: 11077-H08H), c1077

(Bruker, USA). Size distribution was measured by nanoparticle size and zeta potential analyzer (Malvern, UK). Fluorescence lifetime was measured with a F900 fluorescence spectrometer (Edinburgh Instruments Ltd., U.K.). X-ray powder diffractometer was processed by RIGAKU Ultima IV system (Rigaku, Japan). X-ray photoelectron spectroscopic (XPS) was detected by using ESCALAB 250 system (VG, USA). Brunauer-Emmett-Teller (BET) nitrogen adsorption-desorption isotherms were recorded using a ASAP 2020 surface area and porosity analyzer (Micromeritics Instrument Corp, USA).

Preparation of *o*-CDs. *o*PD (0.60 g) was dissolved in absolute ethanol (60 mL) and sonicated for 5 min to make it homogeneous. The mixed solution was transferred to a 100 mL Teflon-lined autoclave and reacted for 12 h under 180 °C, then naturally cooled to room temperature to obtain a brown clear solution, which was further purified through a silica gel column chromatography and the fluorescent component was collected as a pure carbon dots.

Determination of Fe^{2+} and H_2O_2. Different concentrations of Fe^{2+} solution (50 μL) was added into *o*-CDs (100 μL, 20 μg/mL), after reacting for 5 min, the above reaction solution was removed to a fluorescent cuvette and measured by a F4600 fluorescence spectrometer. Following that, 50 μL of H_2O_2 with various concentrations were added into the above system (*o*-CDs+Fe^{2+}) and continued to react for 5 min. Then, the fluorescence intensity of *o*-CDs was quantitatively determined.

Synthesis of the NH_2-functionalized DMSN. DMSN was prepared according to the previous report with minor modifications.[1] Briefly, TEOA (68 mg) was diluted with 25 mL of ultrapure water and stirred at 80 °C for 30 min, followed by adding sodium salicylate (168 mg) and CTAB (380 mg) to the reaction solution. After keep stirring for 1 h, TEOS (4.0 mL) was slowly added dropwise to the above mixed solution, and gently stirring for at least 12 h to obtain a milky white slurry. The suspension was centrifuged at 8000 rpm for 15 min, and the precipitate was repeatedly washed with water and ethanol to remove unreacted precursor. Finally, the obtained product was continuously stirred for 6 h in a mixed solution of HCl and ethanol (HCl: ethanol = 1:9) at 60 °C to remove the template, centrifuged and repeated three times to obtain DMSN, which was dried at 80 °C in a vacuum drying oven.

100 μL APTES was added into the DMSN dispersion solution (10 mL, 10 mg/mL) under magnetically stirring for 12 h to obtain NH_2-functionalized DMSN. The suspension was centrifuged

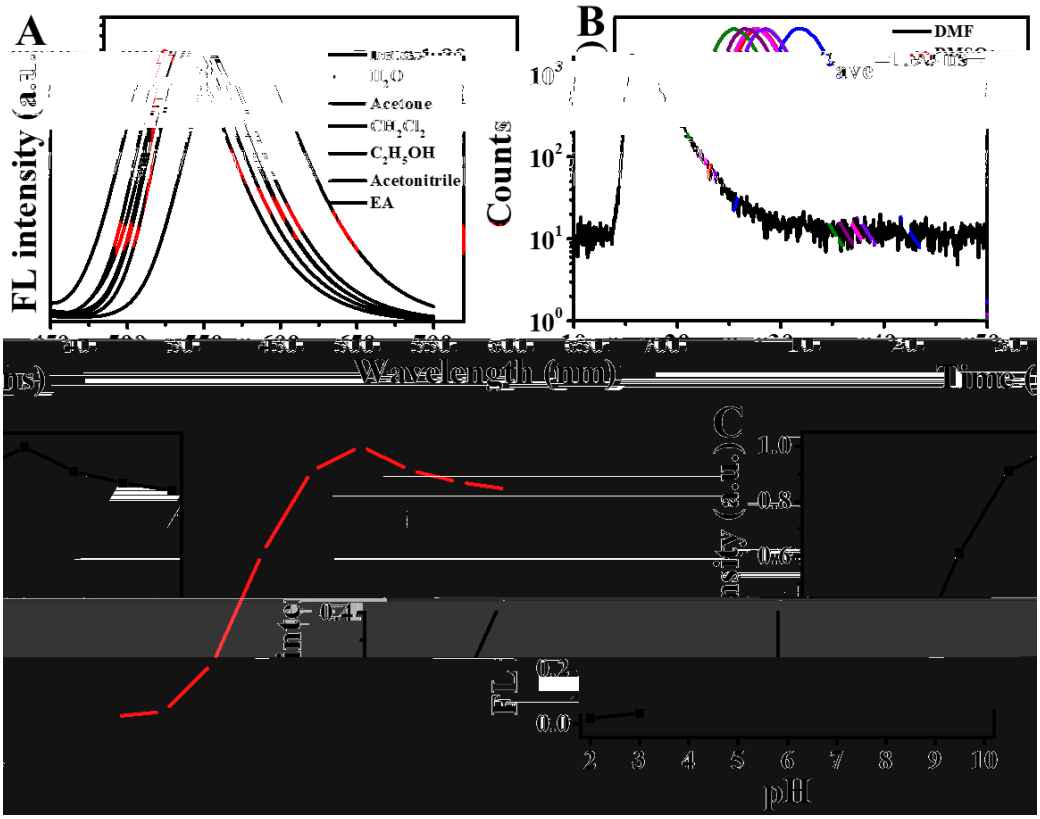

Figure S1. Fluorescence properties of *o*-CDs. (A) Time-resolved fluorescence decay curves. (B) Solvent effect. (C) The fluorescence intensity at different pH.

o-CDs exhibit solvent effect and different color fluorescence in different polar solvents. As the polarity of the solvent increases, the fluorescence emission wavelength gradually bathochromic shift, and the fluorescence gradually transitions from green to yellow (Figure S1B). Besides, as indicated in Figure S1C, the fluorescence intensity of *o*-CDs varies greatly at different pH. As the pH increases, the fluorescence intensity increases significantly and the fluorescence intensity reaches maximum at pH = 7.0, when the pH is greater than 7.0, the fluorescence intensity gradually decreases.

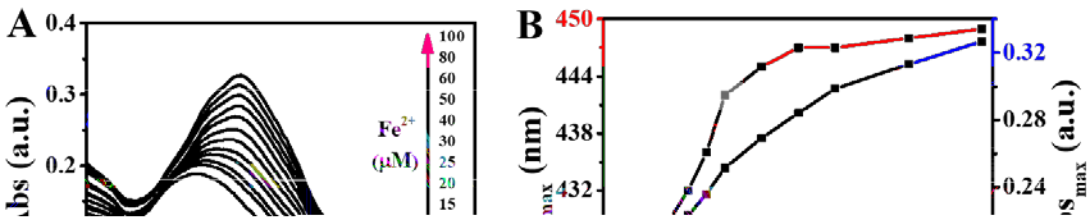

Figure S2. The change of the UV-vis absorption spectrum (A) and absorption wavelength (B) of *o*-CDs in response to Fe^{2+}.

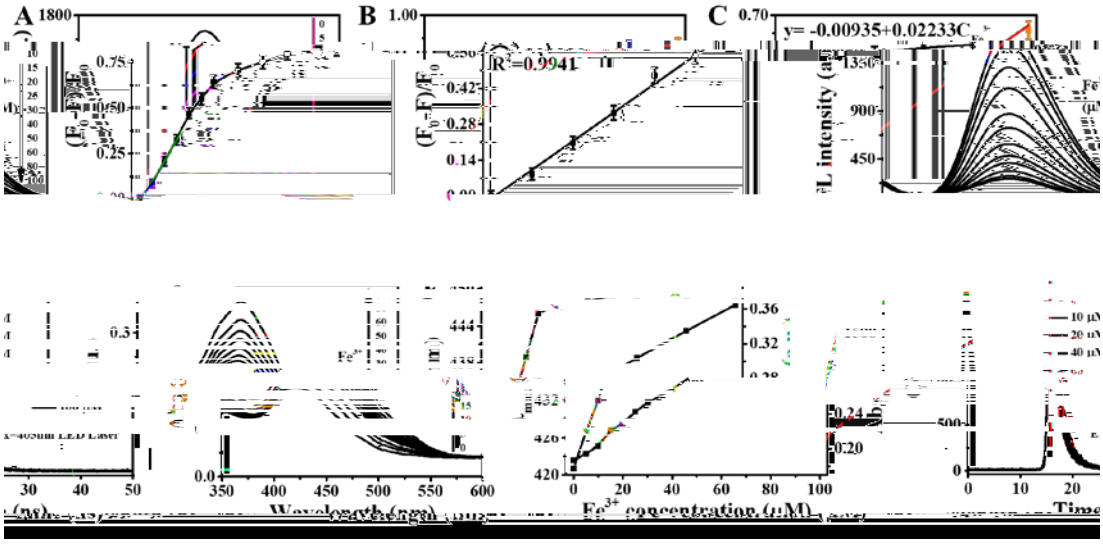

Figure S3. (A) Fluorescence spectra of *o*-CDs adding different concentrations of Fe^{3+} (0 – 100 μM). (B-C) Plot of the fluorescence intensity of *o*-CDs against Fe^{3+} concentration. Time-resolved fluorescence decay curves (D), UV-vis absorption spectrum (E), and absorption wavelength (F) of *o*-CDs versus different concentrations of Fe^{3+}.

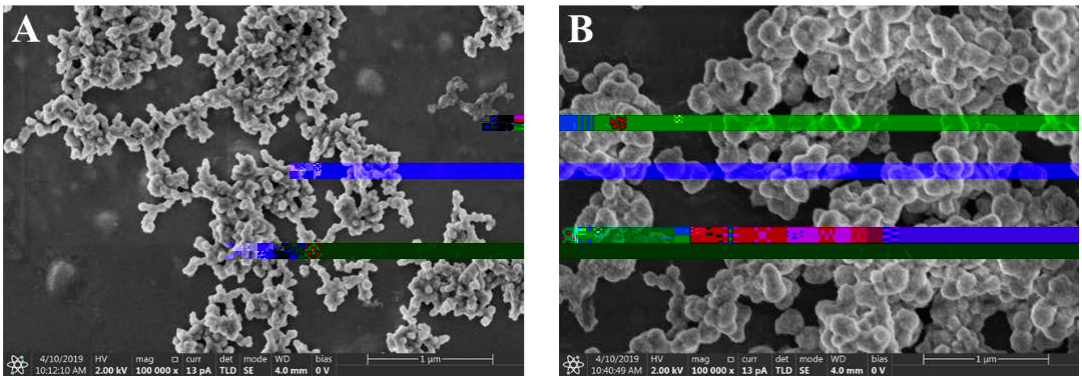

Figure S4. SEM of nanoparticles were formed by the interaction of *o*-CDs and Fe^{2+} (A) and (*o*-CDs + Fe^{2+}) + H_2O_2 (B).

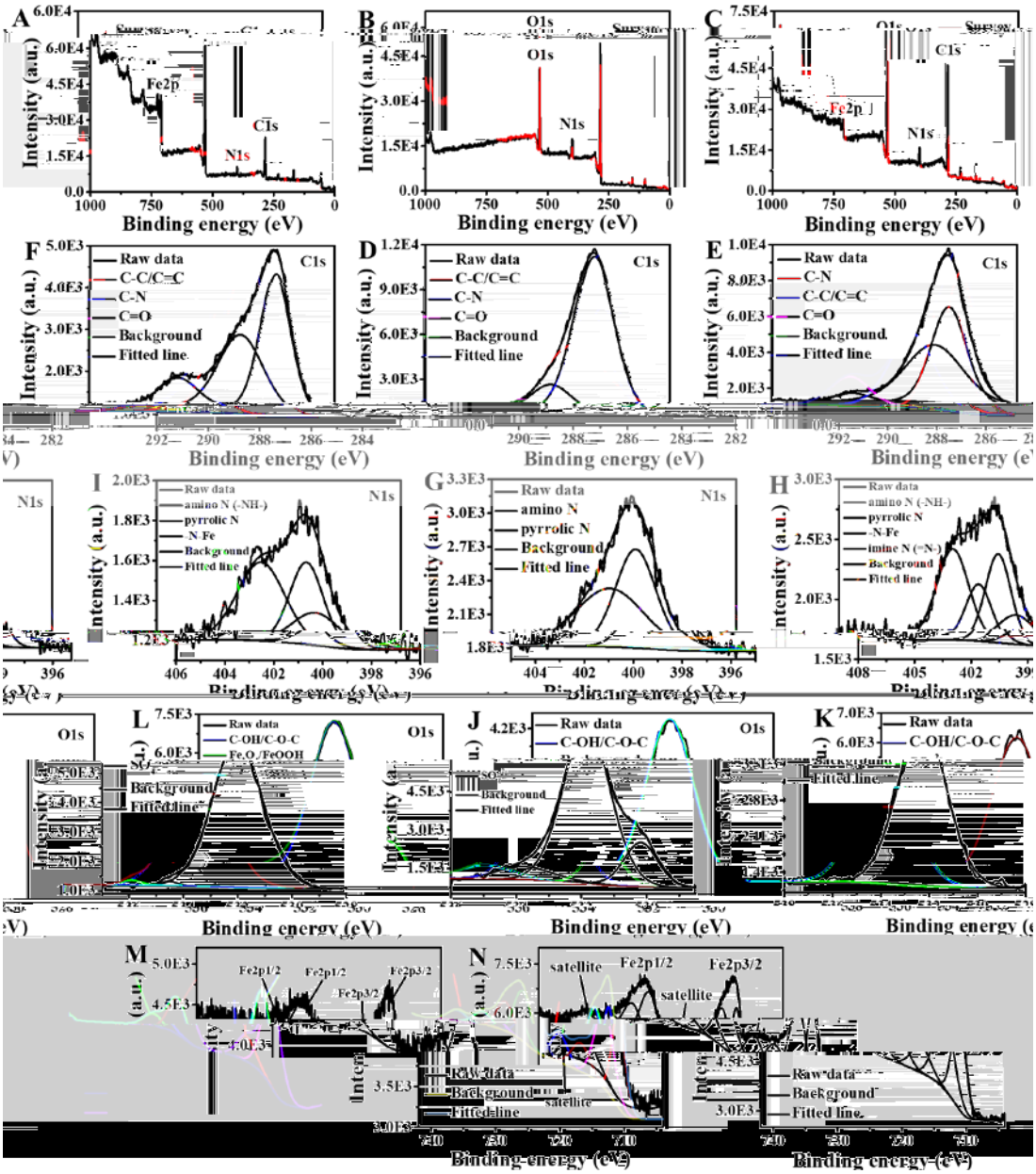

Figure S5. The XPS spectra of *o*-CDs responding to Fe^{2+} and H_2O_2. The survey XPS spectra (A-C), narrow scan spectra of C 1s (D-F), N 1s (G-I), O 1s (J-L), and Fe 2p (M, N) of *o*-CDs.

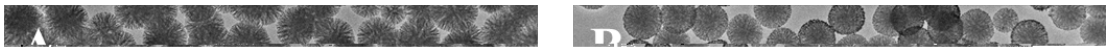

Figure S6. TEM of DMSN (A) and DMSN-Au NPs (B).

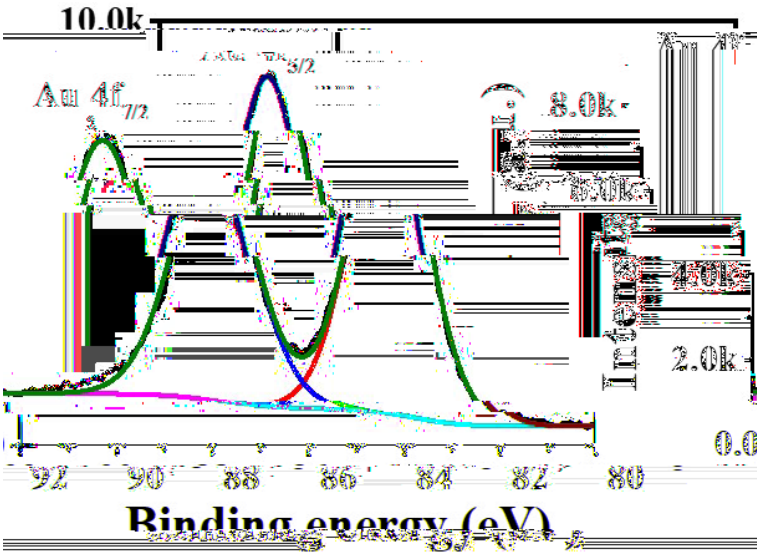

Figure S7. The XPS spectra of Au 4f in DMSN-Au NPs.

Table S1. Comparison of the sensing performance of different methods for detecting CEA.

Method	Linear range (ng/mL)	LOD (ng/mL)	Reference
SERS	1-1000	1.0	[4]
Electrochemical	100-10^5	0.14	[5]
Colorimetric	0.05-100	0.0211	[6]
Chemiluminescence	0.1-64	0.085	[7]
Fluorescence	0.1-80	0.0745	**This work**

Figure S8.

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